Research Article
Cirrhosis and Liver Failure

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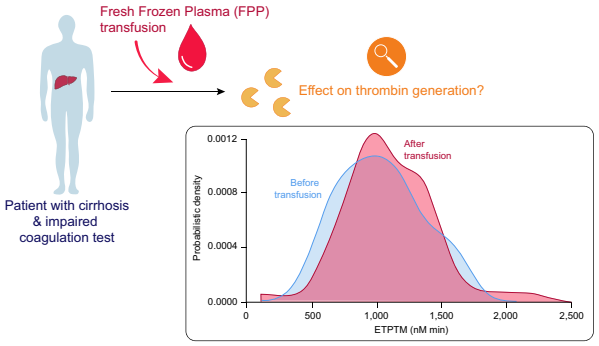
Fresh frozen plasma transfusion in patients with cirrhosis and coagulopathy: Effect on conventional coagulation tests and thrombomodulin-modified thrombin generation

Graphical abstract



> Enhanced thrombin generation & coagulation tests in a limited number of patients
> Slight decrease of thrombin generation in 34% of cases

Highlights

- FFP transfusion before invasive procedures enhanced the total amount of generated thrombin by 5.7%.
- Transfusion slightly decreased thrombin generation in a subgroup of patients, presumably by replenishing protein C.
- Responses to FFP transfusion were similar in patients with compensated/decompensated cirrhosis, ACLF, infection or shock.
- Benefit of FFP transfusion in cirrhosis was too modest to justify its indiscriminate use.

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Lay summary

Transfusion of fresh frozen plasma in patients with cirrhosis only slightly improves coagulation test values in a limited number of patients and even appears to worsen them in a third of cases. Transfusion for the purpose of preventing or treating bleeding events could cause inherent risks and costs without clear benefits.

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Fresh frozen plasma transfusion in patients with cirrhosis and coagulopathy: Effect on conventional coagulation tests and thrombomodulin-modified thrombin generation

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Background & Aims: The efficacy of fresh frozen plasma (FFP) transfusion in enhancing thrombin generation in patients with cirrhosis and impaired conventional coagulation tests has not been sufficiently explored. Thus, we aimed to assess the effect of FFP transfusion on thrombin generation in these patients.

Methods: Fifty-three consecutive patients receiving a standard dose of FFP to treat bleeding and/or before invasive procedures – if international normalized ratio (INR)/prothrombin time (PT) ratio were ≥1.5 – were prospectively enrolled. The primary endpoint was the amelioration of endogenous thrombin potential (ETP) with thrombomodulin (ETP-TM) after transfusion, which corresponds to the total amount of generated thrombin. INR/PT ratio and activated partial thromboplastin time (aPTT) were also assessed before and after transfusion.

Results: FFP enhanced ETP-TM by 5.7%, from 973 (731–1,258) to 1,028 (885–1,343 nM × min; $p = 0.019$). Before transfusion, evidence of normal or high ETP-TM was found in 94% of patients, even in those with bacterial infections. Only 1 (1.9%) patient had ETP-TM values reverting to the normal range after transfusion. Notably, no patients with low ETP-TM had bleeding. The median decrease in ETP-TM was 8.3% and the mean was 12.8% in 18 (34%) patients after transfusion (from 1,225 [1,071–1,537] to 1,124 [812–1,370] nM × min; $p \leq 0.0001$). Similar responses to FFP transfusion were observed in patients with compensated and acute decompensated cirrhosis, acute-on-chronic liver failure, infection or shock. FFP significantly ameliorated INR and aPTT values ($p < 0.0001$), but in a minority of patients the values were reduced to less than the cut-off point of 1.5.

Conclusions: FFP transfusion enhanced thrombin generation and ameliorated conventional coagulation tests to normal values in a limited number of patients, and slightly decreased thrombin generation in 34% of cases.

Lay summary: Transfusion of fresh frozen plasma in patients with cirrhosis only slightly improves coagulation test values in a limited number of patients and even appears to worsen them in a third of cases. Transfusion for the purpose of preventing or treating bleeding events could cause inherent risks and costs without clear benefits.

Introduction

Over the last decade there has been considerable progress in understanding the complex mechanisms behind the coagulopathy of cirrhosis. Landmark studies have demonstrated that there is a substantial balance between pro- and anticoagulant factors in cirrhosis. Despite preserving normal thrombin generation, this balance is relatively unstable and prone to tip towards hemorrhage or thrombosis, depending on the prevailing circumstantial risk factors to which patients are exposed.[1,2] In this process, factor VIII, an endothelial-released coagulation factor (typically increased in cirrhosis) to some extent counteracts the decline of the other procoagulant factors synthesized by the liver. As the levels of the naturally occurring anticoagulants are also decreased in cirrhosis, the anticoagulant effect of protein C, protein S and antithrombin on thrombin generation is attenuated when compared to normal individuals.[3] Indeed, it has been shown *in vitro* that plasma of patients with cirrhosis may generate adequate thrombin amounts when exposed to tissue factor and exogenous phospholipids, provided that plasmatic protein C is activated by its main physiological activator, thrombomodulin (TM).[3–4]

Despite abnormal results of conventional coagulation tests, patients with cirrhosis are not 'auto-anticoagulated', as was previously believed. Conversely, some are at risk of venous thromboembolism when compared to the general population of non-cirrhotic individuals, because the balance may shift toward the production of higher than normal amounts of thrombin and hypercoagulability.[4–6] Conventional coagulation tests such as the prothrombin time (PT) and activated partial thromboplastin time (aPTT) are based on clot formation as an endpoint, which occurs when approximately 5% of thrombin is generated.[7] Therefore these tests are suitable for assessing the procoagulant arm of coagulation, but fail to fully assess its

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anticoagulant counterpart.[4] Furthermore, TM is not added to these tests to mimic the endothelial activation of the protein C pathway. Therefore, PT and aPTT are not reliable indicators of *in vivo* thrombin generation, so they are not suitable for investigating coagulopathy in cirrhosis and guiding transfusion policy.

However, it is still common practice to attempt to correct the deficiency of coagulation factors, suggested by PT/international normalized ratio (INR) and aPTT, with fresh frozen plasma (FFP) transfusions before invasive procedures or to control ongoing bleeding events. This practice is illustrated by a nationwide British study that collected data from 85 hospitals over a 28-day period, which showed that 30% of the patients with cirrhosis were transfused with at least one blood component during their admission to the hospital, and that FFP was prescribed in nearly 30% of cases.[8] Similar figures were observed in a survey in the United States, showing that patients with cirrhosis consumed a disproportionate 32.4% of the units of FFP in a tertiary hospital, despite accounting for less than 8% of total hospital admissions.[9]

FFP has been used for many years on the assumption that patients with cirrhosis are at high risk of bleeding because they have frequent episodes of digestive hemorrhage and prolonged PT and aPTT. However, scarce data are available on the effect of FFP transfusions on thrombin generation to support biological plausibility and indiscriminate use. There are few published studies, and most provide insufficient evidence to make strong recommendations in this regard. Data from an *in vitro* study simulating the effect of transfusion by mixing samples of pooled normal plasma with plasma from patients with cirrhosis suggested that thrombin generation does not appreciably change after the addition of normal plasma, despite PT and aPTT shortening suggesting otherwise.[10] Although the prohemostatic effect was small, a different *in vitro* study showed a difference in response to FFP transfusion in patients with acute-on-chronic liver failure (ACLF) compared to patients with acute decompensation of cirrhosis, compensated cirrhosis and healthy controls.[11] This gives grounds to hypothesize that a very limited number of patients would benefit from FFP transfusion in real life settings, because thrombin generation is already expected to be preserved before transfusion in most patients with cirrhosis.

Our aim was to prospectively assess the effect of FFP transfusion on TM-modified thrombin generation procedures (this corresponds to the total amount of generated thrombin) in patients with cirrhosis and impaired conventional coagulation tests.

Patients and methods

Patients

Patients with cirrhosis who were candidates for FFP transfusion were enrolled in the study. All patients were admitted to the University of São Paulo School of Medicine Hospital between August 2014 and January 2018. Two physicians interviewed and examined all patients prior to transfusion and 6 h after the administration of FFP to assess acute transfusion reactions. Bleeding events were assessed at the end of each procedure and daily for 5 days after inclusion during hospital stay. Any bleeding episode was recorded. Healthcare providers were blinded to the results of the thrombin generation procedure, which was not intended for patient management purposes. Diagnosis of cirrhosis for each patient was made by a panel of experts reviewing all relevant records. These included physical examination, laboratory parameters, presence of signs of cirrhosis and/or portal hypertension on imaging (nodular liver, splenomegaly and/or collaterals), liver biopsy and liver stiffness measurement higher than 12 KPa. Inclusion criteria were: age older than 18 years, cirrhosis of any etiology, INR/PT ratio ≥1.5 and indication for FFP transfusion. We excluded all conditions other than cirrhosis that could interfere with coagulation, including ongoing use of anticoagulant drugs (heparins or any kind of oral anticoagulants) at the time of enrolment, or that had been discontinued less than 7 days before enrolment in the study, advanced malignancy (except for non-melanoma skin cancer), pregnancy or hemostatic disorders other than those pertaining to cirrhosis. Patients with a history of recurrent deep venous thrombosis (except portal vein thrombosis), chronic kidney failure, FFP transfusion during the previous 10 days, and refusal to participate were also excluded. Acute decompensation of cirrhosis and ACLF were defined in accordance with criteria reported in the CANONIC study,[12] and the scores calculated as reported on the website of the European Foundation for the Study of Chronic Liver Failure using the tools available at http://www.efclif.com. Forty-six healthy individuals (median 31.5, range 18–60 years old; 25 females) formed the control group for the procedure of thrombin generation.

Study design

This prospective observational study followed the Strobe Statement.[13] Consecutive adult patients received a standard dose of FFP (10–20 ml/kg) after enrolment to control acute bleeding or as prophylaxis before an invasive procedure if the INR/PT ratio was ≥1.5. Decision on FFP transfusion was made according to the clinical judgment of the attending physician, and dose was determined by considering the dry weight of patients with ascites and/or hepatic hydrothorax. FFP was provided by the institutional blood bank, which is ISO 2001 and American Association of Blood Banks certified for the quality of donor selection, blood collection, processing, storage, transport and distribution of hemocomponents. FFP was separated by centrifugation of whole blood, frozen within 6 to 8 h after collection, and stored at temperatures below −30 °C for up to 24 months.

Efficacy assessment

Primary endpoint was the increase of ETP with TM measured before and after FFP transfusion. Secondary endpoints were: i) amelioration of other thrombin generation parameters and conventional coagulation (INR/PT ratio and aPTT) tests following FFP transfusion; ii) occurrence of bleeding and iii) transfusion-related side effects, defined as any occurring within 6 h after FFP infusion.

Sampling

Personnel involved in drawing blood samples were trained to adhere to a stringent standard operating procedure for control of pre-analytical variables that were considered critical for the thrombin generation procedure, as reported elsewhere.[14,15] Blood samples were collected before and up to 6 h after FFP transfusion, with minimal stasis (tourniquet time less than 1 min), using 21/23 G sized needles into plastic 2.7 ml tubes (*BD Vacutainer®. Franklin Lakes. NJ. EUA)* containing 3.2% buffered sodium citrate (final concentration 0.109 mol/L). Prior to transfusion, the median time for sample collection was 225 [118–765] min for patients undergoing invasive procedures

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and 125 [70–385] min for patients admitted with ongoing bleeding ($p = 0.106$). After transfusion, the median time was 98 [60–195] and 125 [90–240] min ($p = 0.162$), respectively. Platelet-poor plasma (PPP) was prepared within 2 h from collection by centrifugation at 3,000 g for 20 min at 20 °C. Plasma aliquots were stored frozen in capped plastic tubes at −80 °C until testing, which was performed in batch analyses whenever a sufficient number of pre- and post-transfusion patient samples were available.

INR/PT ratio and aPTT

PT testing was performed by the PT-Fibrinogen HS Plus® reagent (HemosIL® Instrumentation Laboratory. Bedford, USA). Results were expressed as INR and PT ratio (ratio of times patient/geometric mean of 40 healthy individuals different from those used as controls for the thrombin generation procedure). aPTT testing was performed by the aPTT-SP® reagent with synthetic phospholipids and silica as activators (Instrumentation Laboratory, Bedford, USA). Results were expressed as ratio of test-to-normal coagulation time. Both tests were performed with an automated coagulometer (ACL Top 500, Instrumentation Laboratory).

Thrombin generation procedure

This procedure is based on the activation of coagulation in PPP by the addition of small amounts of human relipidated recombinant tissue factor, which triggers coagulation in the presence of synthetic phospholipids and calcium chloride.[15] Eighty microliters of PPP were pipetted into the well of a microtiter plate and 20 μl of PPP-reagent (5 pM recombinant tissue factor, 4 μM of synthetic phospholipids) and 20 μl of FluCa, a mixture of calcium chloride and fluorogenic substrate (Stago Inc. Asnieres, France) were added. Tests were repeated in additional plasma aliquot by adding soluble TM (5 nmol/L; Sekisui Diagnostics. Stamford, USA). Testing was performed in triplicate, with pre- and post-transfusion samples. Each patient was tested in the same run to decrease inter-assay variability. Testing was performed using an automated fluorimeter-based method which relies on a low-affinity fluorogenic substrate (Z-Gly-Gly-Arg-AMC) to continuously monitor thrombin activity in plasma, according to the technique developed by Hemker *et al.*[16,17] using a dedicated fluorimeter (Fluoroskan Ascent™. ThermoLabsystem. Helsinki, Finland) equipped with the software Thrombinoscope™ (Sinapse BV. Maastricht, The Netherlands). Readings were automatically recorded and the following parameters of the thrombin generation curve were included: lag time (min), peak thrombin (nM), time-to-peak (min) and endogenous thrombin potential (ETP). ETP is the main parameter of the procedure and is defined by the area under the thrombin generation curve, expressed as nM thrombin concentration × min (nMol × min). ETP represents the net amount of thrombin that any given plasma can generate under the experimental conditions and under the driving force of the procoagulants counteracted by the anticoagulants operating in plasma. The parameter ETP ratio was calculated as the ratio of ETP measured in the presence of TM to absence of TM. ETP ratio represents the resistance to the anticoagulant action of TM and can be considered as an index of hypercoagulability.[3] Median values (interquartile range) in our laboratory for 46 healthy individuals were 784 (528–1,321) and 1,515 (1,296–1,786) nM × min for the test with and without TM, respectively. Mean values were 915 ± 458 and 1,578 ± 363 nM × min, respectively. For the ETP ratio, the median was 0.60 (0.4–0.8) and the mean was 0.6 ± 0.2.

Risk and severity of bleeding after invasive procedures

Each invasive procedure was classified according to the practical guidelines of the European Society of Interventional Radiology,[18] which stratifies the risk of bleeding into 3 classes (low, moderate and significant). Bleeding events were classified according to the Consensus Report from the Bleeding Academic Research Consortium as: inexistent, small (does not lead the patient to seek healthcare outside the previously scheduled times), requiring non-surgical medical intervention, drop of >3 g/dl of hemoglobin or the need for surgical procedure to control bleeding, need to transfuse 5 or more units of red blood cells within <48 h of procedure or fatal event.[19]

Ethics

The study was approved by the Institutional Ethics Board and conducted in accordance with the provisions of the Declaration of Helsinki. Informed consent was obtained from the participants at enrolment.

Statistical analysis

A sample size of 40 patients was estimated by considering a 15% difference (pre- *vs.* post-FFP infusion) of the baseline ETP with TM of 1,042 nM × min (610–1,632) in patients with cirrhosis as previously reported by Tripodi *et al.*[10] The level of statistical significance and power were set at 5% and 0.80, respectively. Considering the shape of our data, we compared the median (interquartile range) values of thrombin generation parameters of patients with cirrhosis with the values of healthy individuals (normal controls). Wilcoxon signed rank, Mann-Whitney *U*, Student's *t* test or ANOVA were used to compare continuous variables, where appropriate. Analysis of covariance (ANCOVA) was performed to assess the effect of bacterial infections and shock on thrombin generation with TM after FFP transfusion. When not otherwise specified, results were expressed as median (interquartile range). $p < 0.05$ was considered statistically significant. Calculations were performed using the software SAS v 9.4 (SAS Institute Inc., 2012), Prism 4 and InStat 3 (GraphPad Inc. La Jolla, CA, USA).

Results

Patients

Two-hundred and seventy-six patients were enrolled in the study, as shown in Fig. 1. Two-hundred and five patients were excluded during the screening phase of the study and 18 were secondarily excluded because they did not reach stable thrombin curves. These 18 patients had ACLF syndrome (n = 15; CLIF-C ACLF score of 12.3 ± 3.2; grade I n = 3, grade II n = 4, grade III n = 8), acute decompensation of cirrhosis (n = 2), active bacterial infections (n = 15) and shock (n = 7).

After exclusions, 53 patients were eligible for analysis. Baseline characteristics of patients are summarized in Table 1. This series was composed mainly of male patients (n = 37; 69.8%) with advanced alcohol-related cirrhosis as the most common etiology (n = 21; 39.6%). The prevalence of active bacterial infection at inclusion was 43.4%. Eight patients (15%) had compensated cirrhosis. Nineteen patients (36%) had acute decompensation of cirrhosis, with a mean CLIF-C AD score of 49.8 ± 13.3 (score ≥60; n = 4). The reasons for decompensation

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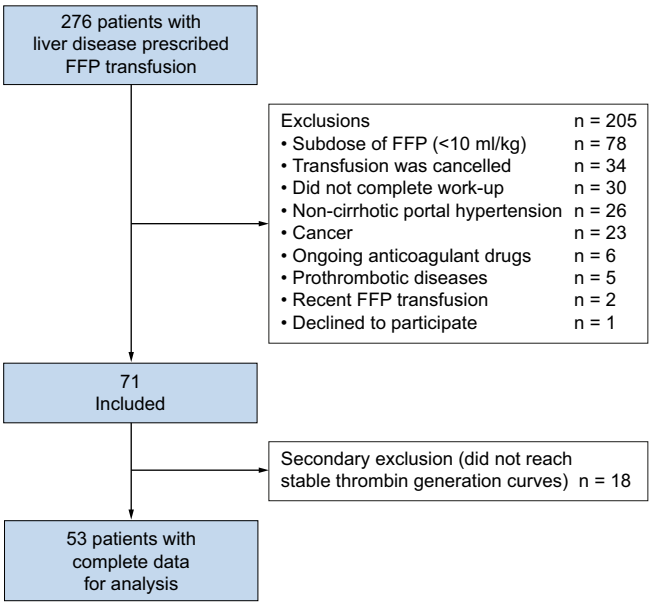

Fig. 1. Enrolment of patients.

Table 1. Baseline characteristics of the patients.

Characteristic	
Age (years)	51.3 ± 11.7
Male	37 (69.8%)
Etiology of cirrhosis	
Alcohol	21 (39.6%)
Viral hepatitis	13 (24.5%)
NASH	8 (15.1%)
Miscellaneous	11 (20.7%)
Child–Pugh classification	
A	1 (1.9%)
B	16 (30.2%)
C	36 (67.9%)
MELD score (points)	24.7 ± 8.1
Compensated cirrhosis	n = 8
Acute decompensation	n = 19 (AD score 49.8 ± 13.30
ACLF	n = 26 (ACLF score 11.2 ± 2.4)
Baseline measurements	
Hemoglobin (g/dl)	8.6 ± 0.4
Platelets ($\times 10^3$/mm^3)	81.4 ± 95.9
Albumin (g/dl)	2.6 ± 0.6
Bilirubin (mg/dl)	6.0 ± 6.7
Creatinine (mg/dl)	1.7 ± 1.4
Fibrinogen (mg/dl)	170 ± 100
Active bacterial infection	23 (43.4%)
Hemodynamic instability (shock)	12 (22.6%)
Procedures	
Low risk of bleeding	9 (33.3%)
Intermediate/high risk of bleeding	44 (66.7%)
Indications for transfusions	
Acute bleeding	25 (47.2%)
Procedures/surgery	28 (52.8%)

Results are expressed as mean/standard deviation or n (percentage).
ACLF, acute-on-chronic liver failure; AD, acute decompensation; MELD, model for end-stage liver disease.

were ascites (n = 9), bleeding (n = 7), bacterial infection (n = 2) or encephalopathy (n = 1).

Twenty-six patients (49%) fulfilled criteria for ACLF, with a mean CLIF-C ACLF score of 11.2 ± 2.4 (grade 1 ACLF n = 8; grade 2n = 9, grade 3n = 9). The precipitating events were bacterial infection (n = 15), bleeding (n = 9), head trauma (n = 1) or unknown (n = 1).

FFP indication and requirements

Twenty-eight patients (52.8%) received FFP prophylactically prior to invasive procedures or surgery, and 25 (47.2%) were transfused in an attempt to treat acute bleeding episodes, of whom 13 also underwent invasive procedures. Most patients underwent procedures with moderate bleeding risk (n = 19; 46.3%). Five (12,1%) underwent high-risk and 17 (41.4%) low-risk procedures. The procedures were: endoscopy (with biopsy n = 1, elastic band ligation n = 2, or with no procedures n = 10), central line placement (n = 4), thoracentesis (n = 3), transparietal liver biopsy (n = 3), transjugular liver biopsy (n = 2), colonoscopy with biopsy (n = 2), paracentesis (n = 2), bronchoscopy (n = 2), subarachnoid hematoma drainage (n = 2), myocardial revascularization (n = 1), abdominal wall drainage (n = 1), cardiac catheterization (n = 1), uterine cervix conization (n = 1), bone marrow biopsy (n = 1), core needle biopsy (n = 1), endoscopic retrograde cholangiopancreatography (n = 1) and transjugular intrahepatic portosystemic shunt (n = 1). All procedures were performed by expert operators. The median time of blood collection was 3 h before and 2 h after transfusion. A total of amount of 210 FFP units (mean of 4 units per patient) was transfused. The mean dose of transfused FFP was 11.3 ± 1.3 ml/kg.

Parameters of thrombin generation in patients with cirrhosis before transfusion

Table 2 shows thrombin generation parameters for patients with cirrhosis and controls. Before transfusion, normal or high thrombin generation was found in the majority of patients. A total of 94% had ETP with TM values higher than the 25th percentile (973 [731–1,258] nM × min) and 96% higher than the lower limit of the mean values (1,008 ± 345 nM × min) of the healthy controls.

Effect of FFP on INR/PT and aPTT ratios

Results of the conventional coagulation tests are shown in Fig. 2 and Table 3. After transfusion, INR/PT and aPTT ratios were significantly ameliorated (Fig. 2) (p <0.0001), corresponding to an improvement of 33.7, 23.5 and 16.6%, respectively. However, correction of INR/PT ratios to values <1.5 was observed in 8 (15%) and 21 (40%) of the patients, respectively. aPTT was already <1.5 at baseline in 41 (77.4%) and was corrected in an additional 10 (18.9%) patients (Table 3).

Effect of FFP on ETP

Fig. 3 and Table 3 show comparison of results (pre- *vs.* post-FFP infusion) for ETP with or without TM. Although there was some between-patient variability, the median levels of ETP with or without TM were increased after transfusion (p = 0.008 and p = 0.019, respectively). Though statistically significant, the relative increment after transfusion was modest, amounting to 5.7%. It is noteworthy that ETP with TM decreased by a median of 8.3% and a mean of 12.8% in 18 (34%) of the patients after FFP transfusion (from 1,225 [1,071–1,537] to 1,124 [812–1,370] nM × min; p ≤0.0001). In all of these patients, baseline values of ETP with TM were already within the range found in controls and remained this way after FFP transfusion. The ETP ratio remained virtually unchanged (from 0.82 [0.79–0.85] to 0.82

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Table 2. Baseline parameters of thrombin generation in patients with cirrhosis and healthy controls.

Thrombin generation	Cirrhosis (n = 53)	Healthy controls (n = 46)	*p* value*	Proportion of patients with normal/high parameters of thrombin generation in cirrhosis**
Lag Time (min)				
with TM	3.7 (3.2–4.8)	4.1 (3.7–4.5)	0.16	26 (49%)
without TM	2.9 (2.4–3.5)	3.5 (3.2–3.8)	0.0001	34 (64%)
Time–to–peak (min)				
with TM	5.7 (5.2–6.8)	6.5 (5.9–7)	0.01	28 (53%)
without TM	5.2 (4.6–5.7)	6.1 (5.67–7)	0.0001	40 (75%)
Peak (nM)				
with TM	154 (119–200)	188 (123–289)	0.01	39 (73%)
without TM	175 (130–221)	295 (230–342)	0.01	10 (18%)
ETP (nM × min)				
with TM	973 (731–1,258)	784 (528–1,321)	0.1	50 (94%)
without TM	1,256 (986–1,526)	1,515.5 (1,297–1,786)	0.0002	23 (44%)
ETP ratio	0.82 (0.79–0.85)	0.60 (0.4–0.8)	0.0001	51 (96%)

Data expressed as median [interquartile range 25-75th] or n (percentage).
ETP, endogenous thrombin potential; TM, thrombomodulin.
* Healthy and cirrhotic populations were compared (Mann-Whitney *U* test, level of significance <0.05).
** Patient were considered normal if their results ≥25th percentile of controls.

[0.77–0.87]; $p = 0.75$). Table 4 shows the effect of FFP transfusion on ETP with TM according to the stage of cirrhosis.

Effect of FFP on the other parameters of thrombin generation

Although there was a significant improvement after transfusion in the peak and time-to-peak of thrombin generation when measured with TM, this corresponded to a change of 23.4% and 10.5%, respectively. Conversely, lag time significantly worsened (Table 3). Table 4 shows the effect of FFP transfusion on other parameters of thrombin generation according to the stage of cirrhosis.

Effect of bacterial infection and shock on thrombin generation

No significant differences were observed in ETP with TM values regarding the presence of active bacterial infection ($p = 0.3395$) or the presence of hemodynamic shock ($p = 0.3730$). Fig. 4 shows thrombin generation modified by TM curves in healthy controls and patients with cirrhosis (whole series, infection, compensated cirrhosis, acute decompensation of cirrhosis and ACLF).

Bleeding events and acute transfusion reactions

Three patients had grade 3 bleeding (need for transfusion) and 2 had grade 2 (drop of 2–3 g/dl in hemoglobin levels). These patients underwent high or moderate risk procedures: subarachnoid hematoma drainage (n = 2), myocardial revascularization (n = 1), colonoscopy with biopsy (n = 1) and drainage of abdominal wall abscess (n = 1). All of them had thrombocytopenia (platelet count range 23,000–56,000/mm^3) and also received platelet transfusions. FFP transfusions corrected the PT ratio – but not the INR – in 4 out of the 5 patients who bled. All of them had ETP with TM within normal values. A significant increase in peak thrombin generation from 127 (122–154) to 182 (158–193) nM was observed in these patients after FFP transfusion (30% change). In contrast, only 2 (3.8%) out of the 53 patients had ETP with TM values below normal, and none of them bled. FFP transfusion corrected thrombin generation in only 1 patient. Five patients (9.4%) had adverse acute reactions related to transfusion (mild allergic reactions, n = 2; transfusion associated cardiac overload, n = 3). One of these patients developed acute pulmonary edema, requiring mechanical respiratory support.

Discussion

Over the last 2 decades, guidelines have been developed to establish rational standards for transfusion of FFP. Although not specifically aimed at patients with cirrhosis, most guidelines adopt arbitrary cut-offs based on INR/PT ratios and/or aPTT prolongation greater than 1.5 times the normal value to trigger FFP transfusion before procedures deemed to increase the risk of bleeding, or in an attempt to stop overt hemorrhage. In this study, we examined the role of FFP transfusion on thrombin generation in patients with cirrhosis and abnormal results of conventional coagulation tests when undergoing invasive procedures or during bleeding events.[20,21] Thrombin generation, especially when modified to include soluble TM, is considered more reliable than the PT or aPTT to represent the balance of coagulation operating *in vivo*.[14] The main finding is that infusion of FFP enhanced ETP with TM by only 5.7% despite the amelioration of the results of conventional tests of coagulation such as PT and aPTT. Although the increase in the total amount of generated thrombin was modest after FFP transfusion, a higher percentage change was observed in peak thrombin. Peak thrombin generation seems to be more sensitive than ETP to variations in coagulation factor concentration after FFP transfusion and sometimes a more sensitive indicator of plasma thrombin-generating capacity. This was observed both in the current and in a previous study of patients with dilution coagulopathy.[22] Although ETP and peak levels are usually well correlated, the peak may theoretically be a more sensitive predictor in assessing hemorrhage risk.[15,22] However, bleeding complications were too rare in our cohort to draw conclusions.

To our knowledge, this has not been previously reported in cirrhosis. Interestingly, evidence of normal or high thrombin generation before transfusion was found in 96% and 98% of patients, as shown by the ETP with TM and ETP ratio (with/without TM), respectively. Only 2 patients (3.8%) had ETP with TM below normal and FFP transfusion corrected thrombin generation in only one of them. Notably, none of them had a bleeding event. Our novel observation is that patients with compensated/acute decompensated cirrhosis or ACLF seem to respond to FFP transfusion in similar ways, with a significant percentage change in peak thrombin values and low increase in the total amount of generated thrombin. It should be emphasized that very sick patients were excluded because they could not reach stable thrombin curves. Furthermore, the current study was not designed to specifically assess differences in

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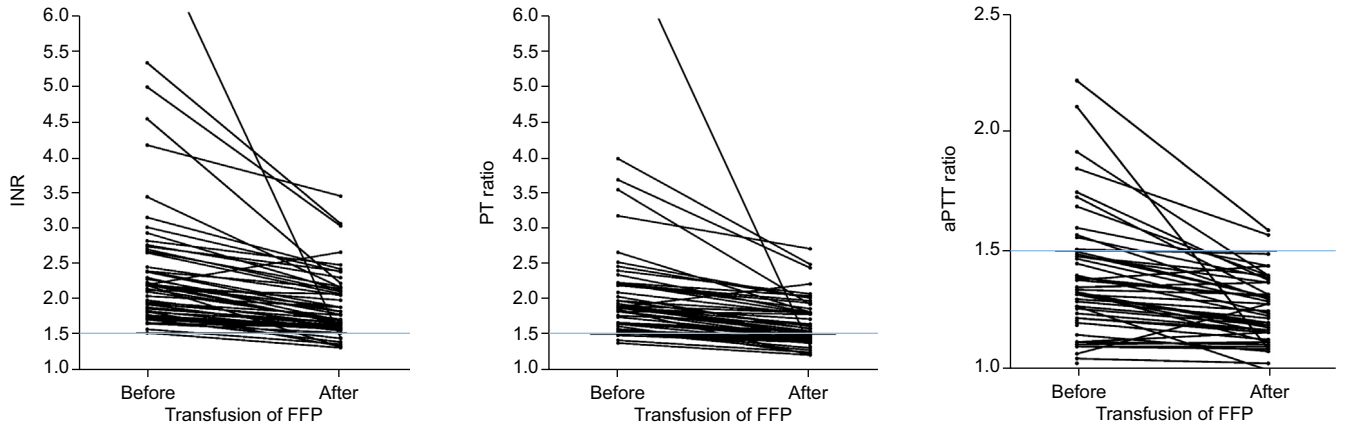

Fig. 2. INR, PT ratio and aPTT values before and after FFP transfusion. Horizontal lines represent the upper limit (1.5) of the normal range for the 3 tests. aPTT, activated partial thromboplastin time; FFP, fresh frozen plasma; INR, international normalized ratio; PT, prothrombin time.

Table 3. Conventional coagulation tests and thrombin generation parameters observed before and after FFP transfusion in the whole casuistic.

	PPF transfusion			
	Before	**After**	**Percentage change (%)**	***p* value**
Conventional tests				
INR	2.2 (1.7–2.6)	1.7 (1.6–2.1)	−33.7	< 0.0001
PT ratio	1.9 (1.6–2.2)	1.6 (1.4–1.8)	−23.5	< 0.0001
aPTT ratio	1.3 (1.2–1.5)	1.2 (1.1–1.3)	−16.6	< 0.0001
Thrombin generation				
ETP (nM × min)				
with TM	973 (731–1,258)	1,028 (885–1,343)	5.7	0.019
without TM	1,256 (986–1,526)	1,327 (1,142–1,626)	5.7	0.008
ETP ratio	0.82 (0.79–0.85)	0.82 (0.77–0.87)	1	0.87
Lag time (min)				
with TM	3.7 (3.2–4.8)	4.2 (3.5–5.1)	13.5	0.02
without TM	2.9 (2.4–3.5)	3.1 (2.7–3.7)	6.9	0.02
Peak (nM)				
with TM	154 (119–199.5)	190 (155–234.5)	23.4	<0.0001
without TM	174.8 (129.5–220.5)	208 (173–250)	19	<0.0001
Time–to–peak (min)				
with TM	5.7 (5.2–6.8)	6.3 (5.6–7.1)	10.5	0.05
without TM	5.2 (4.6–5.7)	5.3 (4.9–5.8)	1.9	0.2

Data expressed as n (percentage) and as median [interquartile range]. Wilcoxon signed rank test, level of significance <0.05. ETP, endogenous thrombin potential; ETP ratio, ETP with/without TM; FFP, fresh frozen plasma; TM, thrombomodulin.

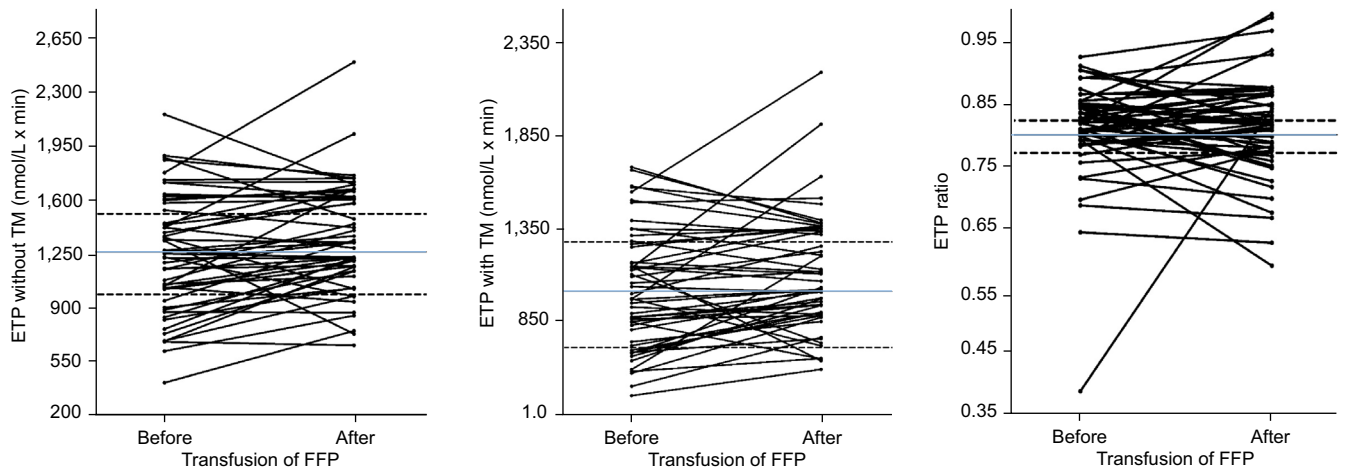

Fig. 3. ETP without TM, ETP with TM and ETP ratio before and after FFP transfusion. Continuous lines represent the median and dot lines represent the interquartile range. ETP, endogenous thrombin potential; FFP, fresh frozen plasma; TM, thrombomodulin.

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Table 4. Thrombin generation with thrombomodulin parameters observed before and after FFP transfusion in different clinical scenarios of cirrhosis.

	FFP transfusion			
	Before	After***	Percentage change	*p* value
Healthy controls (n = 46)				
ETP (nM × min)	784 (528–1,321)	–	–	–
Lag time (min)	4.1 (3.7–4.5)	–	–	–
Peak (nM)	188 (123–289)	–	–	–
Time–to–peak (min)	6.5 (5.9–7.0)	–	–	–
Compensated cirrhosis (n = 8)				
ETP (nM × min)	1,138 (871–1,461)	1,108 (881–1,356)	–2.7	0.461
Lag time (min)*	3.8 (3.2–3.9)	4.2 (4.1–5.4)	10.5	0.032
Peak (nM)	177 (144–230)	212 (177–287)	18.6	0.023
Time–to–peak (min)*	5.4 (4.9–5.8)	6.2 (5.5–6.4)	14.8	0.008
Acute decompensation of cirrhosis (n = 19)				
ETP (nM × min)	1,057 (685–1,393)	1,131 (940–1,364)	7.0	0.069
Lag time (min)*	3.3 (2.7–4.2)	3.8 (3.1–6.3)	15.1	0.125
Peak (nM)*	154 (110–187)	187 (133–201)	21.4	0.118
Time–to–peak (min)*	5.6 (5.0–6.5)	6.7 (5.6–6.7)	19.6	0.042
ACLF (n = 26)				
ETP (nM × min)	960 (709–1,143)	999 (881–1,241)	4.1	0.050
Lag time (min)	4.5 (3.3–5.9)	4.4 (3.8–5.3)	–2.2	0.349
Peak (nM)	160 (119–206)	189 (154–242)	18.1	0.020
Time–to–peak (min)	6.3 (5.3–8.0)	6.4 (5.6–7.5)	1.6	0.449
Undergoing invasive procedures (n = 30)				
ETP (nM × min)**	960 (711–1,330)	1,031 (940–1,343)	7.4	0.093
Lag time (min)	4.0 (3.6–4.8)	4.3 (3.8–5.2)	7.5	0.096
Peak (nM)	179 (123–212)	192 (160–291)	7.3	0.013
Time–to–peak (min)	5.8 (5.2–6.8)	6.2 (5.5–6.9)	6.9	0.125
Treatment of bleeding episode (n = 23)				
ETP (nM × min)**	1,000 (503–1,163)	976 (766–1,362)	–2.5	0.1467
Lag time (min)	3.4 (2.9–4.6)	4.2 (3.4–5.3)	23.5	0.085
Peak (nM)	152 (114–265)	189 (149–225)	24.3	<0.0001
Time–to–peak (min)	5.8 (5.1–7.1)	6.3 (5.6–11.2)	8.6	0.217

Data expressed as median [interquartile range].
* p <0.05 *vs.* controls (Mann-Whitney *U* test).
** p = 0.689 comparison of patients undergoing invasive procedures × bleeding episode (Mann-Whitney *U* test).
*** Wilcoxon matched pairs test for comparison before and after FFP transfusion. ETP, endogenous thrombin potential; FFP, fresh frozen plasma.

response according to the phases of cirrhosis. These findings need to be confirmed in future clinical studies.

The doses of infused FFP (at least 10 ml/kg) in this study were within the range recommended as appropriate by most current guidelines.[23–25] Even so, the observed effect was modest and limited to a few patients. Therefore, the beneficial effect of FFP on thrombin generation in clinical practice could be even smaller than that observed in this study if one considers that audits have revealed that a substantial number of patients are usually transfused with a dose of <10 ml/kg.[26,27]

The preservation of thrombin generation in cirrhosis is presumably due to rebalanced coagulation brought about by the concomitant deficiency of procoagulants (except factor VIII and von Willebrand factor) and naturally occurring anticoagulants. This results in an acquired resistance to the anticoagulant action of TM owing to increased levels of factor VIII and decreased levels of its main physiological inhibitor, protein C.[3,28] In this regard, our findings that thrombin generation at baseline was preserved are in line with the current understanding of the coagulation process in cirrhosis. Perhaps FFP infusion would make the balance between procoagulants and anticoagulants more stable. However, FFP transfusion may also slightly decrease thrombin generation, presumably by transfusing naturally occurring anticoagulants. Indeed, we observed an increased lag time and a significant reduction of the total amount of generated thrombin (ETP with TM) following FFP transfusion in more than one-third of our patients, although

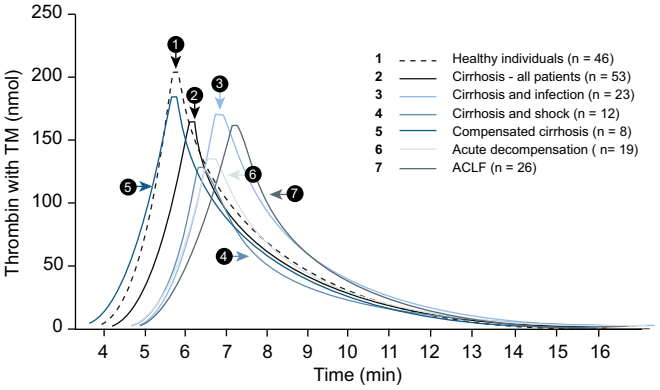

Fig. 4. Thrombin generation modified by thrombomodulin curves in healthy controls and patients with cirrhosis (whole series, infection, compensated cirrhosis, acute decompensation of cirrhosis and ACLF). ACLF, acute-on-chronic liver failure; TM, thrombomodulin.

peak values have improved. One could speculate that FFP transfusion could decrease the normal or high baseline thrombin generation by replenishing protein C, which results in a decreased thrombin generation observed in the subgroup of patients in this study. It should be emphasized that the decrease did not seem to have a detrimental clinical effect because the baseline total amount of generated thrombin was already within the range found in controls and remained this way after

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FFP transfusion. Furthermore, the decrease was within the analytical variation of the test. Although not previously reported in patients with cirrhosis, the observations of this study are in agreement with previous data in critically ill adult patients and neonates showing that FFP infusion results in a concomitant increase in the levels of the naturally occurring anticoagulants antithrombin, protein C and protein S, ultimately leading to reduced thrombin generation.[29,30]

The inclusion of a high number of patients with bacterial infection and shock strengthened the generalization of our findings. Among patients with cirrhosis, those with shock are the most likely to receive FFP prescription in clinical settings. Furthermore, although limited data are available for cirrhosis, the release of endogenous heparinoids by the endothelium during ongoing infections, and the acute coagulopathy subsequent to shock (and protein C activation) would theoretically impair thrombin generation.[31] Our findings suggest that even in patients with cirrhosis and bacterial infections or shock, thrombin generation is preserved at baseline, and these high-risk patients have benefitted little from FFP transfusion to enhance thrombin generation.

Several previous studies for patients with chronic liver disease have shown that FFP transfusion results in normalization of the results of conventional coagulation tests in a small number of patients.[11,32–34] The current study confirms this finding, demonstrating correction of INR/PT ratios to values <1.5 (empirically considered by many guidelines as cut-off values associated with low bleeding risk) in 15% and 40% of patients, respectively.

Taken collectively, the results of the current study on the effect of transfusion on thrombin generation and conventional coagulation tests give grounds to argue against the common practice of using FFP indiscriminately and based on arbitrary INR/PT ratio cut-off values. In fact, this practice, although not endorsed by the AASLD,[35] is still commonly employed, producing substantial costs and increasing risks for patients. In addition, volume expansion with FFP infusion is likely to increase portal pressure and, thus, paradoxically the bleeding risk in these vulnerable patients.[36,37] Despite the evidence of normal or high thrombin generation in cirrhosis, we should acknowledge that the decrease of baseline levels of procoagulant factors and platelet count raises concerns about the management of coagulopathy during invasive procedures or bleeding episodes. A more effective approach than transfusing FFP should include: a) platelet transfusion (count of at least 56,000/mm^3 is critical for thrombin generation[38]), b) fibrinogen administration (reduces the permeability of fibrin clot by 51–63%,[11] improves dysfibrinogenemia[39] and decreases bleeding risk,[40] c) and/or more efficient procoagulant agents such as the infusion of prothrombin complex concentrate, which has more profound procoagulant effects in thrombin generation than FFP, approximately doubling the total amount of thrombin generated.[11] Until the results of future clinical trials become available, the following strategy, based on expert opinion, is advisable: a) patients with stable cirrhosis undergoing invasive procedures or surgery should not receive prophylaxis with FFP or coagulation factor concentrates based on INR/PT ratio values, b) levels of fibrinogen should be replenished to 120–150 mg/dl in case of active bleeding or high-risk procedures, d) prothrombin complex concentrate should be preferred as rescue therapy for patients with active bleeding, e) liberal volume expansion should be avoided due to its adverse effects on portal pressure.

Limitations of this study should be acknowledged. Firstly, we excluded patients with conditions such as chronic renal failure, advanced cancer, pregnancy, history of previous thrombosis or recent transfusion, because they are most likely to suffer from hypercoagulability. Thus, baseline thrombin generation would have been even higher in this subset of patients, and the lack of benefit of FFP transfusion would have been even more striking. Second, the small number of patients with shock limits firm conclusions for this subset of patients. Furthermore, stable and analyzable curves were obtained for all healthy individuals in our cohort and for all patients included in the study, but some patients with cirrhosis did not reach stable thrombin generation curves for unknown reasons, even after repeating tests. This was not related to the storage conditions of the samples or any other pre-analytical variable (which were stringently controlled in our study), but it is in line with other observations reported in literature.[41] Bacterial infection (83%) and shock (39%) were the only remarkable clinical conditions in these patients.

Finally, the relatively small number of observations did not allow us to draw definitive conclusions on the clinical value of FFP transfusion, particularly the effect on different stages of cirrhosis. A large clinical trial for patients with cirrhosis, randomized to receive FFP before invasive procedures, should be carried out to definitively resolve this issue. Such a study had been undertaken under the auspices of the National Institutes of Health, but was prematurely stopped because of insufficient enrolment.[42]

In conclusion, this study has shown that FFP transfusion enhanced thrombin generation in a very limited number of patients with cirrhosis, even though it did ameliorate conventional coagulation tests (PT and aPTT) to some extent. This was due to normal or high thrombin generation in nearly all patients at baseline, even in those with infection, in shock, with acute decompensation or ACLF. Furthermore, transfusion slightly worsened thrombin generation in a number of cases without increasing the risk of bleeding. Perhaps the thrombin generation procedure could identify patients with low thrombin generation, who may benefit from FFP transfusion. Future randomized studies aimed to establish the value of this laboratory procedure to guide transfusion in patients with cirrhosis are warranted. Until then, physicians should choose wisely, and the decision on FFP transfusion should be made on a case-by-case basis and not on arbitrary cut-off values derived from conventional coagulation laboratory tests.

ACLF, acute-on-chronic liver failure; AD, acute decompensation; aPTT, activated partial thromboplastin time; ETP, endogenous thrombin potential; FFP, fresh frozen plasma; INR, international normalized ratio; MELD, model for end-stage liver disease; PPP, platelet-poor plasma; PT, prothrombin time; TM, thrombomodulin.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

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Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Amanda Bruder Rassi: study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; statistical analysis; obtained funding; Elbio Antonio d'Amico: study concept and design; critical revision of the manuscript for important intellectual content; study supervision; obtained funding.

Armando Tripodi: study concept and design; critical revision of the manuscript for important intellectual content; final approval of the version to be published.

Tania Rubia Flores da Rocha: study concept and design; critical revision of the manuscript for important intellectual content; study supervision.

Beatriz Yuri Migita: collection, assembly of data; administrative support.

Caroline Marcondes Ferreira: collection, assembly of data; administrative support.

Flair José Carrilho: administrative, technical and material support; obtained funding; Alberto Queiroz Farias: study concept and design; analysis and interpretation of data; statistical analysis; study supervision, obtained funding; critical revision of the manuscript for important intellectual content; final approval of the version to be published.

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Supplementary data

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